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19. ABSTRACT (Continue on reverse if necessary and identify by block number) Laser Raman multichannel spectrometry, and Fourier Transform (FT)-Raman techniques were evaluated and applied to the analysis of low levels of inorganic nutrients in seawater. The best results were obtained in analyzing nitrite in seawater with an azo-dye derivative of nitrite, flow injection sampling, and combined surface-enhanced Raman scattering (SERS) with resonance Raman scattering (RRS), i.e., SERRS, technique using silver colloids. The limit of detection of the azo-dye complex of nitrite with visible laser excitation and the SERRS technique is estimated to be < 1 nm in basic (pH > 11) seawater solutions and < 12 nm in the acidic (pH ~ 3) solutions. We also investigated near-infrared (NIR) laser excited SERS spectra of an azo-dye derivative of the NO ₂ ⁻ ion by mixing the solution with gold colloid and using FT-Raman			
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19. Abstract (cont.)

spectrometry. The background fluorescence caused by dissolved organic matter in seawater was considerably reduced in the spectrum; however, the present limit of detection with this technique is 20 nM.

We analyzed a depth profile (60-250 m) of nitrite in offshore seawater sampled collected from station ALOHA using basic solutions of an azo-dye complex of nitrite with the SERRS technique as well as with the standard colorimetric technique. The relative variations in the concentration of nitrite with depth determined with these two techniques were found to be in good agreement. Further studies on the intercalibration of various analytical techniques used for determining low concentration of nitrite in seawater are currently underway.

**FINAL REPORT****ONR GRANT NO.:** N00014-90-J-1771**R & T PROJECT:** 4232035--01**CO-PRINCIPAL INVESTIGATORS:** Shiv K. Sharma and
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SOEST
2525 Correa Road
Honolulu, HI 96822**GRANT TITLE:** Applications of Advanced Multichannel Laser Raman
Spectroscopy to Problems in Marine Chemistry**PERIOD OF PERFORMANCE:** March 1, 1990-December 31, 1990**OBJECTIVE:**

Our primary objective was to examine the feasibility of accurately measuring major inorganic nutrients (nitrite, nitrate, ammonium, phosphate, and silicate ions) in seawater using laser Raman spectrometry. We are seeking the most sensitive optical analytical approach to measure these ions in the laboratory with the intention of employing these techniques at sea. To accomplish this, we defined the following objectives:

- (1) Focus our work on detection of low concentrations of nitrite in water in order to optimize our system and identify problems and limitations.
- (2) In order to minimize fluorescence interference, examine near-infrared (NIR) excited Fourier Transform (FT)-Raman technique for measuring SERS spectra of azo-dye derivatives of nitrite ions on gold colloids.

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- (3) Utilize and improve on a combined Resonance Raman and Surface-Enhanced Raman Scattering (RRS + SERS = SERRS) technique, developed with ONR support, for measuring low concentrations of derivatized azo-dye complex of nitrite in seawater.
- (4) Analyze depth profiles of nitrite in seawater from station ALOHA (100 km north of Oahu) as these new techniques come on-line.

EXPERIMENTAL DETAILS:

Both silver and gold colloids were used for enhancing the Raman signal. Procedures used for preparing these colloids are described below:

Silver Colloids:

Silver colloids were prepared by citrate reduction of silver nitrate solution (Sheng *et al.*, 1986; Taylor *et al.*, 1990). For sedimentation and flow injection studies 6 mL of sodium citrate reductant (4.42×10^{-1} M) were added dropwise over a 1-h period via buret to 1 L of rapidly boiling and stirred silver nitrate (3.83×10^{-3} M) solution. This solution was then boiled for a subsequent hour and the volume was returned to a 1 L volume after cooling. Prior to use, the sol was activated with 17.2 mg NaCl and filtered through a Whatman GF/F glass fiber filter to remove large aggregates (Freeman *et al.*, 1988). The concentrated sol had its absorption maxima at 445.6 nm (Taylor *et al.*, 1990).

Gold Colloids:

Commercially available Au colloids (Poly Sciences, Inc.) were used. Gold colloidal solution was also prepared in the laboratory by adding 1% sodium citrate (50 mL) solution to a boiling 1.41 mM HAuCl₄ solution (500 mL). Boiling was continued for about 1 hr. (Lee and Meisel, 1982).

Sample Preparation:

Standard solutions of nitrite ions were prepared from 20 mM stock solution of sodium nitrite. Distilled water, and seawater, which was collected from upper mixed layer, aged, filtered and UV oxidized, were used for preparing the standard solutions.

The azo derivatives of NO_2^- ions were formed by first reacting the nitrite solution with 50 mM sulfanilamide hydrochloride solution in concentrated HCl solution for 1 minute to form the diazonium ion. It was followed by reacting the solution with 5.4 mM solution of n-(1-naphthyl)-ethylenediamine dihydrochloride for at least half an hour (Grasshoff *et al.*, 1983) to form the azo-dye complex of NO_2^- ions in the solution. The pH of solutions were adjusted to 12M by adding NaOH.

The azo-dye solutions were mixed with equal volumes of silver or gold colloidal solution just before the Raman measurements. Raman measurements were made with the mixtures in 1 cm-path-length disposable plastic cuvet as well, using the flow injection sampling system of Taylor *et al.* (1990). Flow injection with an open stream yielded more reproducible results than did the static method, because of reduction in photo-dissociation of azo-dye complex and better heat dissipation.

Resonance Raman and SERRS Spectrometry with Visible Laser Excitation:

The experiments were conducted at HIG's Raman Spectroscopy Laboratory. For resonance Raman, and for a combined surface-enhanced resonance Raman scattering (SERRS) measurements with visible laser excitation, instrumentation included a Spex Triplate spectrophotograph (Model 1877) and an optical multichannel detector system (OMA III, EG&G Princeton Applied Research), a modified Leitz Ortholux I microscope optically coupled to the spectrometer, and an Ar^+ laser (Spectra Physics Model 2020-05) (Sharma and Urmos, 1987). The detector was cooled to -20°C to minimize the dark counts and samples were analyzed on the stage of the micro-Raman spectrophotograph. The measurements were made in 180° scattering geometry. The 488.0 and 501.7 nm lines of

Ar^+ laser were used for exciting acidic and basic dye solutions, respectively. The laser powers at the samples were 2 mW to 10 mW. Spectra were measured with 2-4 cm^{-1} resolution. Data were acquired with 1- or 2-sec detector exposure and accumulated for 60 or 120 scans by computer. This technique yielded excellent signal-to-noise ratio and complete spectra in 1-4 min.

FT-Raman Spectrometry with Near-Infrared Laser Excitation:

The SERS measurements of azo-dye complex of nitrite in seawater on gold colloids were carried out using BOMEM DA-3 FT-Raman spectrometer. The 1.06μ line of a Nd:YAG laser was used for exciting solution mixtures in a 1-cm-path-length quartz cuvet. The laser power at the sample was ~1 watt, and 180° scattering geometry was used for exciting the sample.

RESULTS:

We improved on our initial flow injection sampling cell as well as our silver and gold colloid formulations for measuring SERS spectra of dilute solutions. We optimized our flow injection sampling cell using 1 micromolar crystal violet (CV) as a model analyte on silver colloids. We observed SERRS enhancement factors of 35,000-62,000 times over resonance Raman spectrum (RRS) of CV without Ag colloids. We have been able to obtain spectra for CV down to 1 picomolar (Taylor *et al.*, 1990).

We also examined near-infrared ($1.06 \mu\text{m}$) excited SERS spectra of pyridine and azo complexes on gold colloids using both commercially available Au colloids (Poly Sciences, Inc.) as well as freshly prepared Au colloids. Freshly prepared Au colloids were found to be more stable during the NIR SERS spectral measurements. With NIR FT-Raman SERS technique the fluorescence background was considerably reduced in the spectrum; however, at present our limit of detection (LOD) of nitrite is 20 nM. We have demonstrated that the NIR SERS on gold colloids with FT-Raman spectrometry method

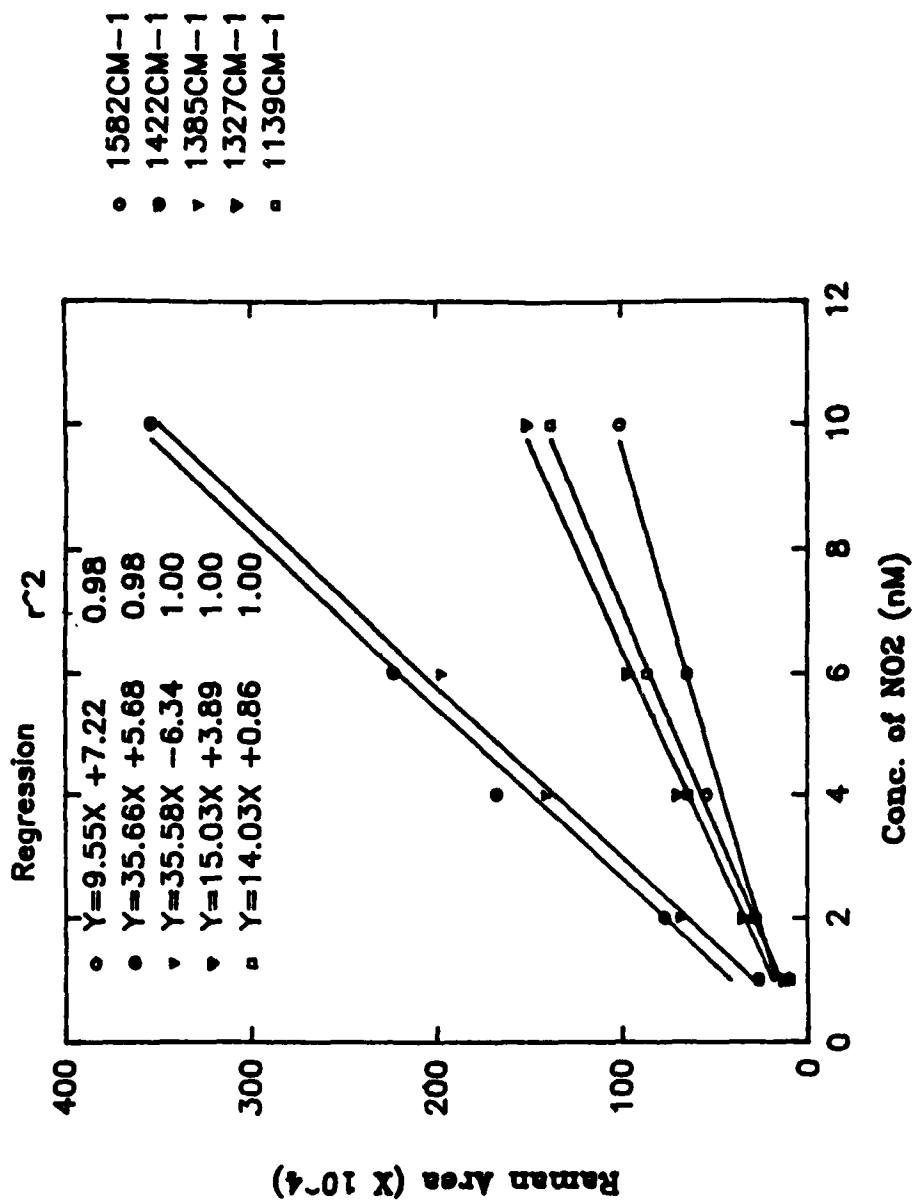


Fig. 1. Standard curve for nitrite in synthetic seawater. Least-square regression for the five SERRS bands at 1582, 1422, 1385, 1327 and 1139 cm^{-1} .

is well suited for determining moderately low concentrations of nitrite in seawater containing high concentrations of fluorophores. We, however, need to lower the LOD for measuring depth profiles of nitrite in offshore seawater.

Using the azo-dye derivatives of nitrite on silver colloids with visible laser excitation (488.0 and/or 501.7 nm), we obtained SERRS spectra of 1 micromolar nitrite in seawater, which are much improved over resonance Raman spectra such as presented by Furuya *et al.* (1980). In the resonance Raman spectra of the azo-dye complex of nitrite in solutions containing $\leq 0.1 \mu\text{M}$ NO_2^- , the signal-to-noise ratio is very poor. On the other hand, the SERRS spectra of solutions of the azo-dye complex of nitrite in nano-molar range can be measured with good singal-to-noise ratio. In an acidic medium ($\text{pH} \leq 3$), our limit of detection of nitrite ion in seawater with the SERRS technique is estimated to be 12 nM. As the intensities of Raman signal of azo-dye derivatives of nitrite are pH-dependent, we investigated the pH dependence of SERRS spectra of the azo-dye derivatives in aqueous solution and found that at high pH (>11) the Raman signal caused by the stable $\text{N} = \text{N}$ vibrational mode shows large enhancement. The limit of detection in synthetic seawater in a basic solution is estimated $\leq 1 \text{ nm}$ (Fig. 1). Given the sensitivity of the technique, extreme care must be taken to avoid unwanted contamination in the reagent, Ag colloids, and flow injection system.

In natural seawater our attempts to analyze the SERRS spectra of azo-dye complex of nitrite in basic solution was initially hampered because of precipitation of Mg^{2+} and Ca^{2+} ions as hydroxides at high pH (>9). Hydroxide precipitation causes rapid coagulation of Ag colloids. If the solution is filtered before mixing with Ag colloids, the azo-dye complex is adsorbed to the precipitate and remains on the filter paper. The problem was overcome by pre-treating this seawater with NaOH and filtering the solutions before the azo-dye derivative reaction occurs. After this treatment one can follow the usual procedure to derivatize nitrite. With this procedure, we are able to detect $\leq 1 \text{ nm}$ nitrite in seawater. We have successfully used pyridine as an internal

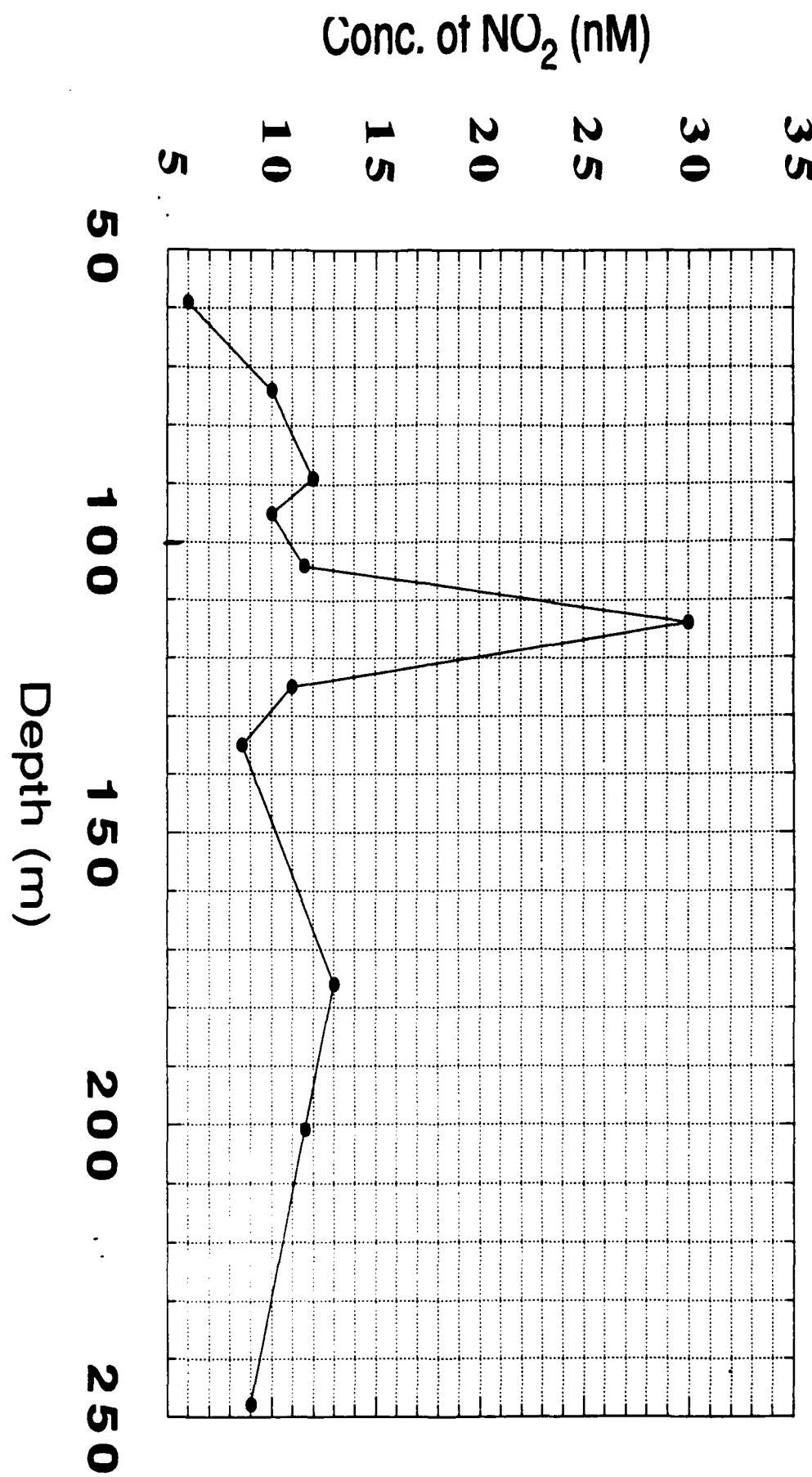


Fig. 2. Depth profile of NO_2 ions in seawater collected from the station ALOHA, 100 miles north of Oahu.

standard in aqueous solutions containing less than 20 nm of nitrite. Use of pyridine as an internal standard helps minimize errors resulting from fluctuation in laser power.

We have analyzed a depth profile (60-250 m) of nitrite in offshore seawater samples collected from station ALOHA, we used basic solutions of the NO_2 azo-dye complex and SERRS technique with 501.7 nm laser excitation as described above as well as the standard photometric (colorimetric) technique. With the colorimetric technique, the nitrite concentrations were determined by measuring the extinction of the dye in acidic solutions at 540 nm. The relative variations in the concentration of nitrite as a function of depth, determined with two techniques, are in good agreement. Nitrite shows a large maximum in concentration in seawater collected from a depth of 115 m. The concentration of nitrite in the seawater from 115 m depth is found to be ~6 times the concentration of nitrite ion in the seawater collected from a depth of 60 m. The absolute concentration of nitrite in these seawater samples determined using SERRS technique is in the range 5-30 nM (Fig. 2); that is ~3 times less than that observed using the standard photometric technique (10-90 nm). Possible causes for this discrepancy are under investigation. One possibility is that some of the nitrite was lost during sample preparation for the SERRS technique. It should, however, be pointed out that below 20 nm nitrite concentration, the accuracy of measurement of SERRS technique is ± 1 nM whereas the accuracy of the photometric method is ± 10 nM.

We are trying to further refine our procedure so that we do not have to filter the seawater before analyses. We would like to use a chelating agent that can react with Mg^{2+} and Ca^{2+} to form stable complexes under high pH condition. We will also evaluate centrifugation as a means of precipitate removal. In this way, we will be able to minimize nitrite loss and contamination problems as well as simplify the procedure for sample preparation.

ACCOMPLISHMENTS:

We have demonstrated the potential of enhanced Raman spectroscopic techniques for analyzing nitrite and dissolved organic matter in seawater. The techniques developed with ONR support have proven to be very sensitive and specific. These Raman techniques offer several advantages: (i) small sample volume (0.1 to 1 mL) are required, (ii) low concentrations can be detected, (iii) spectra can be generated in 1 to 2 min., (iv) inorganic salts do not interfere with the Raman scattering, and (v) the interference from co-existing chromogenic material is not as problematic as in the colorimetric technique. The limit of detection of the azo-dye complex of NO_2^- ions in basic solutions of SERRS spectrum is lower ($\leq 1 \text{ nM}$) than any other existing analytical technique. Further refinements are, however, needed before this technology can be used for routine analysis of nutrient in seawater.

FUTURE PLANS:

- (1) Refine the sample preparation technique to avoid the precipitation of Mg^{2+} and Ca^{2+} ions from seawater.
- (2) Identify the reasons for the differences in the absolute value of nutrients in seawater obtained with the SERRS and the colorimetric techniques.
- (3) Measure depth profiles of nitrites from offshore seawater.

INVENTIONS:

None.

PUBLICATIONS AND REPORTS:

Taylor, G. T., S. K. Sharma, and K. Mohanan (1990) Optimization of a flow injection sampling for quantitative analysis of dilute aqueous solutions using combined

resonance and surface enhanced Raman spectroscopy (SERRS). *Appl. Spectrosc.* 44, 635-640.

Taylor, G. T., S. K. Sharma, and K. Mohanan (1990) Marine nutrient analysis with advanced laser-Raman spectroscopy. ASLO/AGU Ocean Science Mtg., New Orleans. *EOS* 71, 134 (abstract).

Taylor, G. T., and S. K. Sharma (1989) Advanced laser spectroscopy in marine sciences. In D. Henderson (ed.), *Hawaii Inst. of Geophysics Bi-annual Report*, 12-15.

In Preparation:

Sharma, S. K., G. T. Taylor, and K. Mohanan, Combined surface-enhanced Raman and resonance Raman scattering (SERRS) measurements of azo-dye complex of nitrite in seawater. *J. Raman Spectroscopy*.

Sharma, S. K., G. T. Taylor, K. Mohanan, and B. McConnell, Near-infrared surface-enhanced Raman scattering (SERS) study of azo-dye complex of nitrite using gold colloids.

Xi, K., S. K. Sharma, G. T. Taylor, and D. W. Muenow. Detection of low concentrations of azo-dye complex of nitrite in fresh water and seawater with surface-enhanced Raman and resonance Raman (SERRS) scattering techniques.

TRAINING ACTIVITIES:

This project has partially supported one Ph.D. candidate and one M.S. candidate. Mr. K. Mohanan is pursuing a Ph.D. degree in Geology and Geophysics, and Mr. Kang Xi is pursuing his M.S. in Chemistry with emphasis on oceanic nutrients analysis. The grant also provided partial support to a postdoctoral staff.

Women or Minorities - 0

Non-citizens - 2

AWARDS/FELLOWSHIPS:

The University of Hawaii Research Council and the Hawaii Institute of Geophysics provided an award of \$78,000 as matching funds towards the "Acquisition of FT-Raman Instrument for Marine Corrosion, Geochemistry and Materials Research" to match \$118,000 awarded by ONR under the Defense University Research Instrumentation Program (DURIP). These awards permitted the purchase of the FT-Raman instrument for making SERS measurements with near-infrared laser excitation.

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Sheng, R. S., L. Zhu, and M. D. Morris (1986) Sedimentation classification of silver colloids for surface-enhanced Raman scattering. *Anal. Chem.* 58, 1116-1119.

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combined resonance and surface-enhanced Raman spectroscopy (SERRS). *Appl. Spectroscopy* **44**, 635-640.